NON-THERMAL EFFECT OF MICROWAVE TREATMENT ON ENZYME SUSPENSIONS
PART II.: CELLULASE ENZYME ACTIVITY

Erika Lakatos, Attila J. Kovács, Ágnes Szerencsi, Miklós Neményi
University of West Hungary, Institute of Biosystems Engineering
Vár 2., Mosonmagyaróvár, 9200, Hungary
e-mail: lakatose@mtk.nyme.hu; kovacsaj@mtk.nyme.hu; szerencsia@mtk.nyme.hu;
nemenyim@mtk.nyme.hu

ABSTRACT

Renewable fuel production (the second generation bio-ethanol production) is one of the key areas of the EU 7th Framework Programme. One of the challenges in this research area is that the degradation of cellulose materials into glucose is very expensive at the moment. Our research aim was to enhance the activity of cellobiohydrolase enzyme using only physical methods. At the beginning of this research we used D-(+)-cellobiose as substrate. During treatments a special designed inverter type microwave oven was used running on 50 W. In this equipment the temperature of enzyme-substrate solution was increased until 45 °C. As control measurement the suspension was heated up on a hot-plate until 45 °C. After the treatment samples were incubated at 37 °C. Enzyme activity was measured at 505 nm by determining glucose concentration. Based on the results the produced glucose of the microwave treated solution was 20% higher than in the solution heated up on a hot-plate.

1. INTRODUCTION

Renewable fuel production is one of the key areas of the EU 7th Framework Programme. Bioethanol has an increasing importance among bio-fuels. According to the International Energy Agency, cellulosic ethanol could allow ethanol fuels to play a much bigger role in the future than previously thought. Cellulose fibers, the major component in plant cells walls, can be converted to generate ethanol. This process is however still expensive and complicated. (Gray, 2006; László et al., 2007).

Forest and agricultural residues (e.g. corn fiber, wheat straw) or energy crops like energy grass could be used as reasonable raw materials for cellulose based ethanol production (Ohgren, 2007; Gáspár et al., 2008,). Cellulose is the raw material of the future, at the same time the degradation into glucose is very expensive now. This degradation can be carried out either with chemical methods like acid working on high temperature, and perhaps on high pressure or with help of the cellulase enzyme system (Balat et al., 2008; Hu and Wen, 2008). The high cost arises in the first case from the extreme technological conditions and chemicals, while in the second case the price of cellulase (Réczey, 1996).

Environmental pollution caused by chemicals is not negligible; therefore we have focused our experiments on enzymatic cellulose degradation. Our research aim was to enhance the activity of cellulase enzyme by using only physical methods.

Recently, more and more research projects (Kermasha et al., 1993; Tajchakavit and Ramaswamy, 1997) are dealing with the non-thermal effects of microwave. Several different researches attempt to clarify this non-thermal effect, e.g. non-thermal heat-shock

induced by low-intensity microwave fields (Pomerai et al., 2000; Ma et al., 2009). The effects of microwave irradiation on enzyme-catalyzed reactions were also published (Roy and Gupta, 2003).

2. MATERIALS AND METHODS

At the beginning of this research a specially designed PANASONIC NNF 653 WF inverter type domestic microwave oven with a FISO MWS-4 fiber optic thermometer was used for microwave treatments running on 50 W at 2.45 GHz (Lakatos, 2006). The power was continuously emitted by the inverter type oven (in contrast to most of the commercial ovens where the microwave power is pulsated). The treatment parameters were adjusted by a computer connected to the oven. Hence individual programming could be carried out. The inside temperature changes the treated materials were followed by the in-built fiber optic thermometer that measures temperature inside microwave field based on Fabry-Perot interferometry (Datta and Anantheswaran, 2001). The homogeneous microwave field i.e. even temperature distribution (Figure 1) was ensured based on our previous examinations (Lakatos et al., 2005). In our experiment D-(+)-cellobiose (Sigma-Aldrich) was used as substrate and 1,4-(1,3:1,4)-B-D-Glucan-4glucano-hydrolase (Sigma-Aldrich, ATCC 26921) in a pH 4.6 buffer solution as the enzyme.

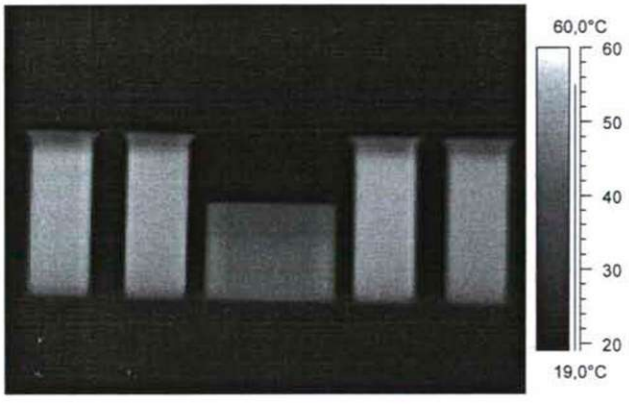

Figure 1. Infrared image of the sample holder can (middle) and the four water trap containers. (Source: Neményi et al., 2006.)

The increase of glucose content referring for enzyme activity changes were measured directly and every 30 minutes after the treatments by using Hitachi UV/Vis spectrophotometer. The duration of increased enzyme activity level was also studied. The treated suspensions were stored in refrigerator at 8 °C for 48 and 96 hours. After this time period the suspensions were heated up on a hotplate up to 37 °C then, the glucose content was measured by spectrophotometer in 30 minute intervals.

During our examinations a question aroused whether the effect of microwave radiation influences the enzymes, the buffer solution or both (Szerencsi et al., 2009). Henceforth, only the buffer solution was heated by microwave and on hotplate. After this heating the enzyme and substrate were added to the pretreated buffer solution, then the glucose content

of samples was detected. In this case the non-thermal microwave radiation could only affect the buffer solution.

The aim of the examination was to reveal the non-thermal effect of microwave irradiation on change of cellulose enzyme in the suspension.

3. RESULTS AND DISCUSSION

In our equipment the temperature of enzyme-substrate solution was increased only until 45 °C for 45 min. (The microwave treatment in this temperature is still considered to be non-thermal.) The control suspension was heated up on a hot-plate until 45 °C for 45 min. After this treatment the samples were incubated on 37 °C.

Directly after the treatment and during incubation in 30 min intervals the enzyme activity was measured at 505 nm (Figure 2).

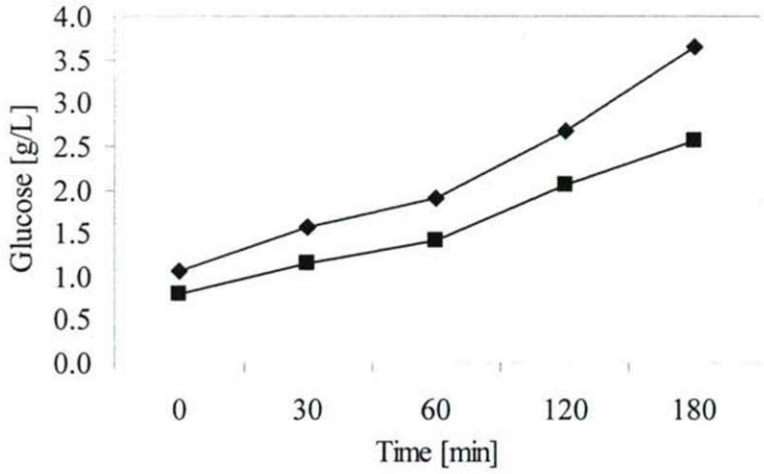

Figure 2. The increased cellulase enzyme activity after microwave (◆) and conventional heat treatment (■) in enzyme substrate suspension.

As the result shows the enzyme activity of microwave treated solution was 20% higher than in the solution heated up on a hot-plate. Significant differences were found between the microwave treated and the heat plate heated (control) samples.

In the long duration experiment we compared enzyme activity changes in stored and in freshly prepared samples (Figure 3).

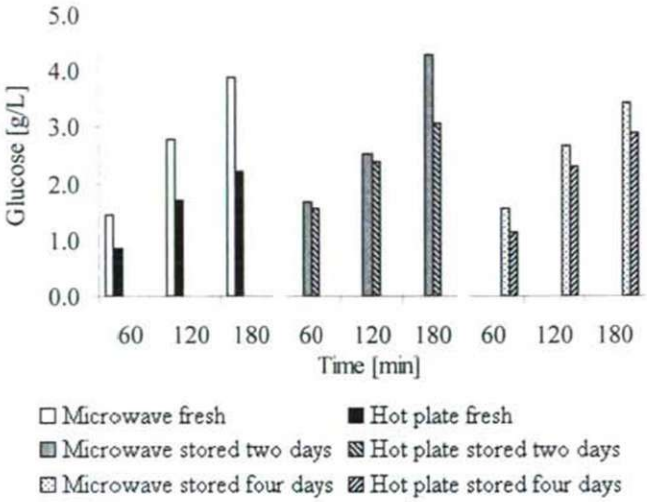

Figure 3. The changed cellulase enzyme activity of microwave and conventional heat treatments; directly after treatment, 48 hours and 96 hours later.

The microwave treated enzyme suspension still has increased activity after 96 hours of treatments.

Figure 4 shows the results of experiments, where only the buffer suspension was treated and the enzyme and substrate were added hereafter. Directly after treatment and during incubation in every 30 minutes the glucose content was measured. In this case the differences were smaller between microwave and conventional heating methods.

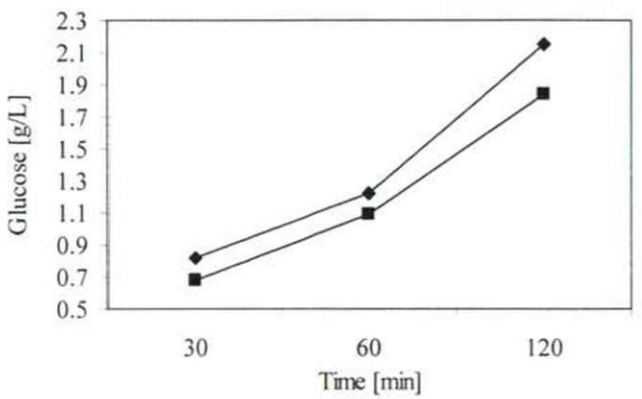

Figure 4. The increased cellulase enzyme activity after microwave (◆) and conventional heat treatment (■) in buffer suspension.

4. CONCLUSIONS

Cellulase enzyme systems have fundamental importance in second generation bioethanol production. Based on our results it can be concluded that the electron magnetic radiation of microwave treatment of enzyme suspension increases the cellulase enzyme activity. This activity still remains after 96 hours and also can be detected if only the buffer solution is treated. The enzyme activity can be significantly enhanced by microwave treatment, so it could be exploited in case of bioethanol production.

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